THE EMBRYO SAC OF PHYSOSTEGIA[1]

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(WITH PLATES VI AND VII)

The material of *Physostegia virginiana* (L.) Benth., upon which the present work is based, was collected near Alma, Michigan, in August 1909. Although the investigation has brought out no new point of fundamental importance, the results are deemed worthy of record.

The ovule arises from the floor of the sporangial chamber as a small protuberance, which in growing pushes out the ovary wall in such a manner that it becomes completely surrounded by the latter except at the funiculus. At the time when the archesporium is distinguishable as a single hypodermal cell, the young ovule is slightly curved, and as growth proceeds this curving becomes more pronounced, until finally an anatropous condition is reached. A single massive integument is developed.

The archesporial cell, which cuts off no parietals, grows rapidly, and is markedly elongated at the time when its nucleus goes into synapsis preceding the first division (fig. 1). This cell, which, on account of the occurrence of the heterotypic prophases in its nucleus, is to be regarded as the megaspore mother cell, by two successive divisions gives rise to a row of four megaspores (fig. 2). Of these the outer three degenerate (fig. 3), while the innermost enlarges and gives rise to the embryo sac.

The nucleus of the functioning megaspore divides, and the two daughter nuclei take up positions near opposite ends of the sac, which becomes strongly curved, and, owing to rapid growth, develops a large central vacuole (fig. 4). Each nucleus divides, forming the four-nucleate stage (fig. 5). These four nuclei by one further division give rise to eight, and walls soon form, resulting in their organization into a typical egg apparatus, three antipodal

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cells which soon multiply to several, and two free polar nuclei (fig. 6).

Shortly before the division to form eight nuclei, a laterally directed lobe begins to develop from the antipodal region of the sac, and at the eight-nucleate stage is very conspicuous (fig. 6). It rapidly invades the integumentary tissue, forming what may for convenience be called the "endosperm lobe," since it is soon to contain nearly all of the endosperm formed. During these early stages it probably serves in a haustorial capacity, as does the greatly enlarged antipodal portion of the embryo sac of *Saururus* (JOHNSON **7**).

Meanwhile the micropylar polar nucleus migrates to the narrow portion of the sac near the antipodals, where it meets and fuses with the polar nucleus of the antipodal group. The resulting fusion nucleus is invariably found in this position (fig. 7).

At about this time the antipodal cell which lies nearest the sac cavity takes on an appearance different from that of the others. It becomes binucleate, the cytoplasm changes in character, staining more deeply, and rapid enlargement causes its wall to become strongly convex (fig. 7). This enlargement continues until the cell bulges out conspicuously into the embryo sac cavity (fig. 10), and its wall thus partitions off the small pocket in which it lies with the other antipodals. In stages somewhat later it bears much resemblance to the first few cells of the endosperm, but the possibility that it also is of endospermous origin is precluded by the fact that it has been observed side by side with an undoubted endosperm nucleus resulting from the triple fusion (fig. 9).

The function of the cell in question is in all probability haustorial, recalling the behavior of the basal antipodal in several genera of the Galieae (LLOYD **10**), although in the sac under consideration the neighboring tissue is not actively invaded. It soon fills all the space formerly occupied by the other antipodals, which disorganize and completely disappear (figs. 13, 14, 16), while in its general form and relation to the vascular supply it is especially well suited to the performance of a nutritive function during the rapid development of the endosperm. Later it disappears and the tissue of the region becomes irregularly broken down (figs. 18–20).

The great variety in form and behavior exhibited by antipodal cells, together with haustorial structures of many types, has been so well summarized (COULTER and CHAMBERLAIN 3) that further comment here is unnecessary, since *Physostegia* offers nothing essentially new.

At the time of fertilization the general aspect of the embryo sac, together with its position in the ovule and its relation to the vascular supply, are as shown in fig. 8. The usual configuration of the egg apparatus is that figured here, but in other cases it exhibits considerable variation from this. In regard to the positions of the nuclei and vacuoles, the synergids represented in fig. 7 show striking similarity to the egg, and it is conceivable that at least the larger one might function as such.

The pollen tube, which has grown down the style into the sporangial chamber, makes its way around the stalk of the ovule, or at times directly over its summit, to the micropyle, through which it enters the embryo sac. Clear cases of fusion of the male nucleus with that of the egg were not observed, but the presence of the pollen tube within the sac, the disorganization of the synergids, the immediate elongation of the egg with divisions to form an embryo, and a triple fusion in the central region of the sac (fig. 9) make it reasonably safe to conclude that fertilization of the usual type occurs.

The formation of the endosperm is of considerable interest. It is initiated by the division of the endosperm nucleus, which occurs in the narrow region of the sac near the haustorial antipodal, as shown in fig. 10. The spindle has a transverse orientation and is very broad, owing to the large number of chromosomes present. The division is accompanied by a longitudinal wall running through the middle of the sac, as shown in fig. 11, which represents a sac cut in a plane at right angles to that of fig. 10. Here the wall is still in process of formation, spindle fibers being evident at its extremities. Extension continues until it comes into contact with the sac wall at or near the end of the endosperm lobe (fig. 12), while in the micropylar lobe it was not observed to do so, and probably ends freely. The nuclei now lying in the two resulting parts of the embryo sac divide, forming transverse

walls (fig. 12), and further similar divisions give rise to a large-celled, thin-walled tissue which fills the endosperm lobe (fig. 13). This endosperm formation may cease abruptly at the narrow portion of the sac (fig. 14), but usually extends for a little distance into the micropylar lobe (fig. 16). The two-ranked arrangement so conspicuous in the endosperm lobe in fig. 13 and in the micropylar lobe in fig. 16 is doubtless due to the longitudinal separation of the embryo sac into two parts as described above.

The cessation of endosperm formation at an indefinite point results in nuclei being left free in the cytoplasm of the micropylar portion of the sac (fig. 13). These nuclei, usually two in number, enlarge (fig. 14) and may occasionally divide, the walls which appear on the spindle fibers being evanescent. Often the nuclei were observed fusing. Consequently, from one to at least four may be present in stages somewhat later, but they play no further active part, and disorganize with the other contents of the micropylar lobe (figs. 18 and 19).

In embryo sacs which show a wall at the first division of the endosperm nucleus it is usual for the sac to be thereby separated transversely into two chambers, and for endosperm to be formed only in the micropylar one. Among such cases the endosperm may pass through a free nuclear stage, as in *Sagittaria* (SCHAFFNER 12), *Limnocharis* (HALL 5), and *Ruppia* (MURBECK 11); or walls may be formed at all of the divisions, as in *Ceratophyllum* (STRASBURGER 13) and the *Nymphaeaceae* (COOK 1 and 2). Less frequently both daughter nuclei resulting from the division of the endosperm nucleus take equal parts in the direct formation of cellular endosperm, as reported for *Peperomia pellucida* (JOHNSON 8), *Heckeria* (JOHNSON 9), and *Datura laevis* (GUIGNARD 4). From the above account it is seen that essentially this is the mode of endosperm formation in *Physostegia*, and in this sac the main point of interest lies in the fact that the first wall is longitudinal rather than transverse. The factors governing the orientation of the spindle and the consequent position of the wall are not at all clear, and the feature is probably best regarded as a minor peculiarity rather than a character of much significance.

The restriction of endosperm to the antipodal portion of the

embryo sac has been observed in a number of cases (COULTER and CHAMBERLAIN 3), the condition reaching its extreme in *Loranthus* (HOFMEISTER 6 and TREUB 14), in which scarcely more than the lower one-tenth of the sac becomes filled with permanent endosperm tissue. Among the Labiatae the work of TULASNE (15), HOFMEISTER (6), and VESQUE (16) shows this to be the prevailing condition in several genera. In *Stachys sylvatica* TULASNE figures endosperm developing in the antipodal region of a slightly curved sac, but without the presence of a special chamber; and in *Betonica* a condition which may well represent a later stage in the same situation. The figures of HOFMEISTER indicate that in *Lamium* the endosperm lobe is well developed before fertilization, as in *Physostegia*. Although no antipodals and only two "Keimbläschen" are represented, HOFMEISTER has figured stages which correspond approximately to those shown in figs. 7, 8, 14, and 16 of this paper.

In all of these cases the embryo is brought into contact with the endosperm by the great elongation of the micropylar cell of the proembryo. The earliest clearly observed stage in *Physostegia* is shown in fig. 13. Here the first division in the fertilized egg has occurred, and the micropylar cell by its great elongation is pushing the chalazal cell into the endosperm, the cells of which at this time are relatively few in number. Nearly all the elongation is accomplished by the one cell, but this soon divides to several (figs. 14 and 16).

The first division in the chalazal cell is longitudinal (fig. 15), as is also the second. Each of the four resulting cells is then divided into two by a transverse wall (fig. 16), and the subsequent divisions proceed with much regularity (figs. 17 and 18).

At the time when the embryo becomes imbedded in the endosperm, the micropylar and endosperm lobes are approximately equal in size. The former, as has been noted above, disorganizes and in later stages becomes entirely obliterated, while the latter increases rapidly in size owing to the active growth of the endosperm. This growth is accomplished at the expense of the cells of the integument, which in the mature seed is recognizable as only one or two layers of cells (figs. 19 and 20). At the same time the

embryo grows rapidly, becomes characteristically dicotyledonous, and displaces nearly all of the endosperm. It attains a length of nearly 2 mm. in the mature seed, the coat of which is formed from the ovary wall.

Summary

1. The archesporium of *Physostegia* consists of a single hypodermal cell, which, without formation of parietals, functions as the megaspore mother cell.

2. The megaspore mother cell by two successive divisions gives rise to a row of four megaspores; the chalazal one enlarges and gives rise to the embryo sac, while the other three disorganize.

3. The mature embryo sac contains an egg, two synergids, three antipodal cells which multiply to several, and two polar nuclei which fuse.

4. During the formation of the embryo sac a lobe develops from near its chalazal end, so that the sac consists of two distinct parts joined by a narrower portion.

5. Double fertilization of the usual type in all probability occurs.

6. The endosperm is cellular from the beginning, the wall accompanying the first division of the endosperm nucleus being longitudinal through the sac. The chalazal portion of the sac, or "endosperm lobe," becomes completely filled with endosperm tissue, which invades and destroys nearly all of the integument; while the micropylar portion of the sac never contains more than a very few endosperm cells, and later disorganizes, becoming completely obliterated by the encroaching endosperm.

7. The first division in the fertilized egg is transverse, and the chalazal cell, which becomes imbedded in the endosperm through the great elongation of the micropylar cell, develops very regularly into a typically dicotyledonous embryo, which displaces nearly all of the endosperm.

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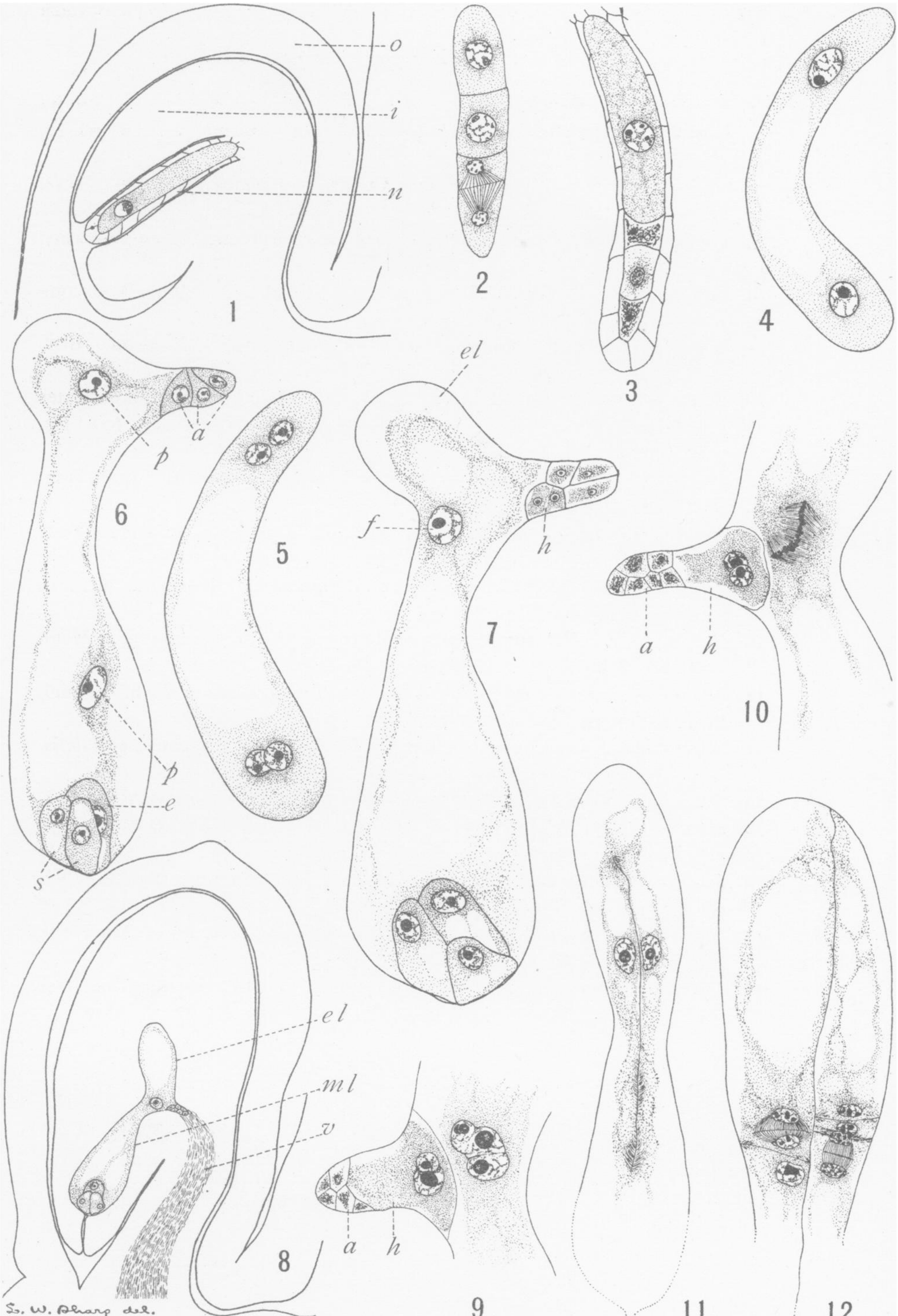

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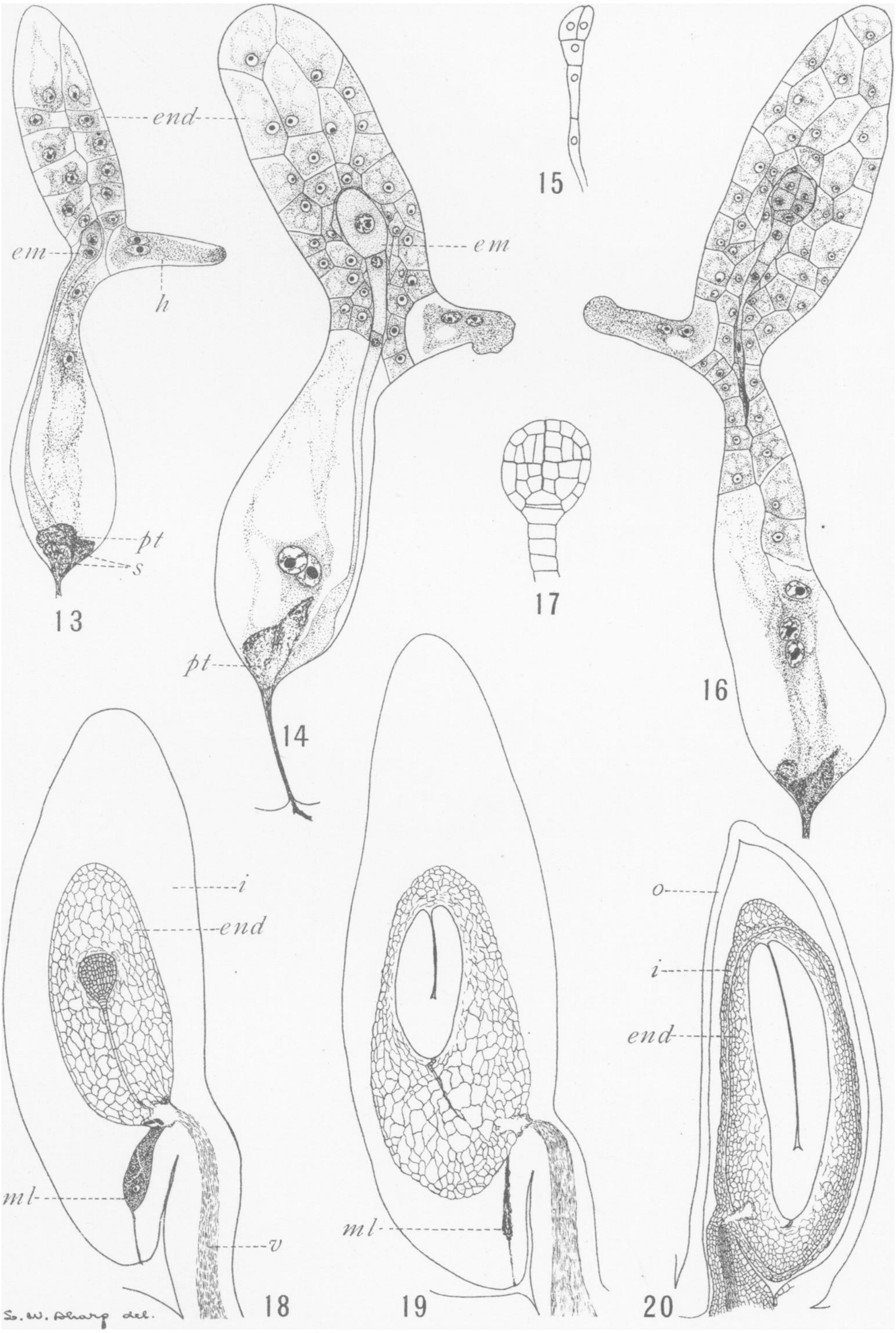

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EXPLANATION OF PLATES VI AND VII

All figures were drawn with the aid of an Abbé camera lucida, have a corresponding orientation, and show magnifications as follows: figs. 1, 11, 13–17, ×215; figs. 2–5, 9, ×462; figs. 6, 7, 10, 12, ×385; fig. 8, ×81; figs. 18, 19, ×45; fig. 20, ×25. The following abbreviations are used: *a*, antipodals; *e*, egg; *el*, endosperm lobe; *em*, embryo; *end*, endosperm; *f*, fusion nucleus; *h*, haustorial antipodal; *i*, integument; *ml*, micropylar lobe; *n*, nucellus; *o*, ovary wall; *p*, polar nucleus; *pt*, pollen tube; *s*, synergids; *v*, vascular supply.

PLATE VI

FIG. 1.—Young ovule with archesporial cell nucleus in synapsis.

FIG. 2.—Four megaspores; the division to form the two micropylar ones is delayed.

Fig. 3.—Four megaspores; the innermost one much enlarged, the other three disorganizing.

Fig. 4.—Two-nucleate embryo sac.

FIG. 5.—Four-nucleate embryo sac.

FIG. 6.—Eight-nucleate embryo sac.

FIG. 7.—Mature embryo sac; the polar nuclei have fused and the antipodals have increased in number; one antipodal beginning to differentiate as a haustorial cell.

FIG. 8.—Ovule with mature embryo sac.

FIG. 9.—Triple fusion of male and polar nuclei; haustorial antipodal cell present; other antipodals disorganizing.

FIG. 10—First division of endosperm nucleus.

FIG. 11.—Daughter nuclei resulting from first division of endosperm nucleus; the wall accompanying the division is still in process of formation.

FIG. 12.—Subsequent endosperm divisions, all accompanied by walls.

PLATE VII

FIG. 13.—Two-celled proembryo coming into contact with the endosperm; haustorial antipodal cell well developed; the other antipodals and the synergids have degenerated.

FIG. 14.—Later stage; endosperm formation has ceased abruptly at the narrow portion of the sac in this case.

FIG. 15.—Chalazal cell of proembryo divided longitudinally.

FIG. 16.—Somewhat later stage; the endosperm has extended into the micropylar lobe.

FIG. 17.—Later stage of embryo.

FIG. 18.—Endosperm invading integument; embryo digesting endosperm; haustorial antipodal cell and micropylar lobe disorganized.

FIG. 19.—Later stage.

FIG. 20.—Section of nearly mature seed; the embryo has used up the greater part of the endosperm, which in turn has obliterated nearly all of the integument; seed coat formed from the ovary wall (*o*).